Journal of Infection and Public Health (2016) xxx, xxx–xxx

http://www.elsevier.com/locate/jiph

Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study

Amin A. Aqel[a,b,*], **Panagiota Giakkoupi**[c], **Hamed Alzoubi**[a], **Ibrahim Masalha**[d], **Matthew J. Ellington**[e], **Alkiviadis Vatopoulos**[c]

[a] *Microbiology and Immunology Department, Faculty of Medicine, Mu'tah University, Al-Karak 61710, Jordan*
[b] *Al-Ghad International Colleges for Applied Medical Sciences, Madinah Branch, Saudi Arabia*
[c] *Department of Microbiology, National School of Public Health, Athens, Greece*
[d] *Medlab Referral Department, Jabal Amman, Amman, Jordan*
[e] *Public Health England, Antimicrobial Resistance and Healthcare Associated Infection Reference Unit, National Infection Service, 61 Colindale Avenue, London NW9 5EQ, UK*

Received 9 November 2015; received in revised form 9 February 2016; accepted 20 February 2016

KEYWORDS
Carbapenemase;
CTX-M;
NDM;
OXA-48-like;
K. pneumoniae;
Jordan

Summary Little is known of carbapenemase producing *Klebsiella pneumoniae* (CPK) in Jordan. This study aimed to determine the prevalence of CPK in a major hospital in Amman, Jordan in 2012–2013 and to characterize the isolates and detect the types of carbapenemase(s) they produced.

For the 296 isolates investigated, species identification and antimicrobial susceptibilities were determined (Vitek II, bioMérieux). Isolates with decreased ertapenem susceptibility were tested for carbapenemase production using the Modified Hodge Test. Isolates with a carbapenemase-positive phenotype were characterized further via multiplex PCRs for extended-spectrum β-lactamase and carbapenemase genes and by Pulsed Field Gel Electrophoresis (PFGE).

Seven of 296 *K. pneumoniae* isolated in 2012–2013 (2.4%) were carbapenemase producers, five produced class D carbapenemases (OXA-48-like) and two produced a NDM metallo-beta-lactamase. All seven isolates also encoded CTX-M enzymes; CTX-M-1-like enzymes were detected in five isolates (two co-producing NDM enzymes and

* Corresponding author at: Mu'tah University, Faculty of Medicine, Microbiology Department, P.O. Box 7, Mu'tah, 61710 Al-Karak, Jordan. Tel.: +962 3 238 6287x3208; fax: +962 3 239 7180 00.
E-mail address: aminaqel@hotmail.com (A.A. Aqel).

http://dx.doi.org/10.1016/j.jiph.2016.02.002

Please cite this article in press as: Aqel AA, et al. Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study. J Infect Public Health (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.002

three co-producing OXA-48-like enzymes), CTX-M-9 was found in the two remaining OXA-48-like producers. PFGE revealed five genetically distinct types amongst the seven carbapenemase producing *K. pneumoniae*, with two pairs of identical isolates associated with patients treated on the same wards.
The emergence of OXA-48-like and NDM carbapenemases associated with multi-drug resistant (MDR) isolates in Jordan is concerning. The strict implementation of infection control practices will help to disrupt the spread of MDR carbapenemase producers in Jordanian hospitals.

Introduction

Carbapenem antimicrobials are the drugs of choice for severe infections caused by multidrug resistant Gram-negative bacteria [1]. Carbapenem-resistant *Enterobacteriaceae* (CRE) can cause treatment failure and constitute an important public health problem [2]. Resistance due to the production of a carbapenemase is horizontally transferred amongst *Enterobacteriaceae* and the types of carbapenemase are myriad; clavulanic-acid-inhibited β-lactamases (Class A: KPC, NMC, IMI, SME, and GES); metallo-β-lactamases (Class B: IMP, VIM, NDM, GIM, SPM, and SIM) and expanded-spectrum oxacillinases (Class D: OXA-48-like) [3].

European surveillance data have indicated the incidence of carbapenem-resistant *Klebsiella pneumoniae* to be low (less than 0.5%) in many Northern European countries including Finland, Denmark, Ireland and Sweden in 2014. By contrast Italy and Greece had endemic problems with frequencies of 34.3% in Italy and 59.4%, respectively (*EARS-Net interactive database: http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/table_reports.aspx) [4]. Elsewhere, carbapenemase-producing Klebsiella (CPK) are considered endemic in the North East of the USA, Puerto Rico, Zhejiang Province of China, Southern Asia, and parts of the Middle East, while very few cases have been reported from Australia and Africa [5]. In Jordan and other Arabian countries the isolation rates for CPK range between 2% and 10% [6—8] and OXA-48 and NDM-1 enzymes are known to predominate amongst *Enterobacteriaceae* in Morocco, Palestine and the Arabian Gulf countries [6,7,9]. While Jordanian hospitals, including our own, do receive medical cases referred from other Arabian countries, little is known of the prevalence of CPK infections, their outcomes and the degree of spread amongst hospital patients [8]. This pilot study aimed to determine the frequency of infections due to CPK and to characterize the isolates and the carbapenemase enzymes they encode in order to determine the molecular epidemiology of CPK amongst effected patients in a major Jordanian teaching hospital.

Materials and methods

Bacterial isolates

From March 2012 to April 2013, 296 *K. pneumoniae* were isolated from clinical specimens (blood culture, urine, fluid, wound, tissue and sputum) taken from 296 patients attending Islamic Hospital which is a 450 bed hospital in Amman, Jordan. Isolates were identified and tested for susceptibility to a panel of antimicrobials (Vitek II, bioMérieux), the results for the latter were interpreted according the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2014 [10].

Screening for carbapenemase producing isolates

Isolates with decreased susceptibility to cefoxitin MIC ≥16 μg/ml, ceftazidime MIC ≥8 μg/ml, cefotaxime MIC ≥2 μg/ml, imipenem MIC >1 mg/l, or meropenem MIC >1 mg/ml were investigated for carbapenemase activity [11]. Also, isolates with decreased susceptibility to ertapenem (zones of 21 mm or less around a 10 μg ertapenem disk) were investigated as possible carbapenemase producers according to CLSI guidelines (2014) [10]. The Modified Hodge Test (MHT) [11] was performed on suspected carbapenemase producers according to CLSI guidelines (2014) [10], with *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-1706 used as positive and negative control strains, respectively.

Please cite this article in press as: Aqel AA, et al. Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study. J Infect Public Health (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.002

Multiplex-PCRs for β-lactamase and carbapenemase genes

Crude DNA lysates were prepared by suspending a 1 μL loop-full of freshly cultured cells in 200 μL of sterile distilled water. The suspension was incubated for 5 min at 95 °C and 1 μL of supernatant from the centrifuged lysates used as template DNA for PCR. Genes encoding CTX-M extended spectrum β-lactamases were detected via multiplex PCR [12], carbapenemase gene families (bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{OXA-48-like}$) were determined according to Giakkoupi et al. [13].

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed to determine the clonal relationships between the isolates using CHEF-DR III apparatus from Bio-Rad Laboratories (Richmond, CA, USA). Briefly, chromosomal DNA embedded into SeaKem Gold Agarose (Lonza, Rockland, USA) was digested overnight with *Xba*I (New England Biolabs, MA, USA). Digested DNA was electrophoresed in 1% agarose (Biorad, CA, USA) at 6 V/cm for 20 h; the pulse time was increased from 5 to 55 s. DNA was visualized and imaged under UV light after staining with GelRed dye (Biotium, San Francisco, USA). PFGE bands in the gel image were detected in Gel Compar II software (v. 3.1b) (Applied Math, Kortrijk, Belgium) and the banding patterns of each isolate compared using the unweighted pair group method with arithmetic averages clustering method by using the Dice coefficient.

Results

Seven of the 296 *K. pneumoniae* (2.8%) were carbapenemase producers (CPK). The isolates were recovered from various specimens from three Jordanian, two Syrian and two Yemeni patients (Table 1). The isolates were all resistant to amoxicillin/clavulanic acid, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefotaxime, cefepime, ciprofloxacin and gentamicin in addition to the carbapenems. Susceptibility to tetracycline (42.8%), amikacin (28.5%) and trimethoprim/sulfamethoxazole (28.5%) varied between the isolates. All seven isolates were susceptible to tigecycline and colistin (Table 1).

PCR amplification of the carbapenemase, and CTX-M extended-spectrum β-lactamase genes revealed that two of the seven CPK harbored a bla_{NDM} gene and five harbored a $bla_{OXA-48-like}$ gene (Table 1). Both of the isolates that had a bla_{NDM} gene also harbored a group 9 bla_{CTX-M} gene (CTX-M-9-like). Likewise, two of the five isolates with a $bla_{OXA-48-like}$ gene also harbored a group 9 bla_{CTX-M} gene, while the remaining three isolates with a $bla_{OXA-48-like}$ gene had a group 1 CTX-M ($bla_{CTX-M-1-like}$) ESBL. All isolates were negative for bla_{VIM}, bla_{IMP} and bla_{KPC}. Phenotypic inhibition tests were in accordance with the molecular findings (data not shown).

The seven CPK isolates were classified into three unrelated PFGE types (A, B, C) with similarity percentages <80%. Type A was further divided to subtypes (A1, A2, A3) (Fig. 1). Types A1 (isolates 2 and 3) and A3 (isolates 7 and 8) represented pairs of PFGE identical isolates that were isolated from different patients (from Jordan, Yemen and Syria) that had were treated on the same wards in our hospital.

Discussion

Extended-spectrum β-lactamases (ESBLs) are produced by half of the *K. pneumoniae* isolates in Jordan [14] which has driven the widespread use of carbapenems. Despite the high value of carbapenems as an empirical treatment against serious infections due to ESBL producers, recent work has shown that 5.6% of the Gram-negative isolates in Jordan were carbapenem resistant [8]. Here, our finding of carbapenemase production in 2.8% of 2012—2013 isolates is lower than published rates in Europe or the USA, but does agree with reported carbapenemase rates from other Arabian countries [6,15]. Furthermore, we identified multiple NDM producers as well as a predominance of OXA-48-like producers, indicating that Jordan faces, at the very least, the same level of challenge to carbapenems as other countries in the Arabian Gulf as well as the wider region (e.g. Palestine, Lebanon, Egypt and Turkey) [6,9,16,17]. The methods we used for phenotypically detecting carbapenemase producers were according to the published standards that were current at the time [11]; however, updated methods for the detection of metallo-enzyme types as well as for OXA-48-like enzymes will improve case ascertainment and thus the determined rate of carbapenemase producers still further. Nevertheless, the identification of these two distinct epidemic enzyme types highlights the urgent need for continued study and surveillance of carbapenemase producers in Jordan, not least to improve the poor ascertainment of carbapenemase producers that is associated with standard front-line diagnostic laboratory workflows which do not specifically

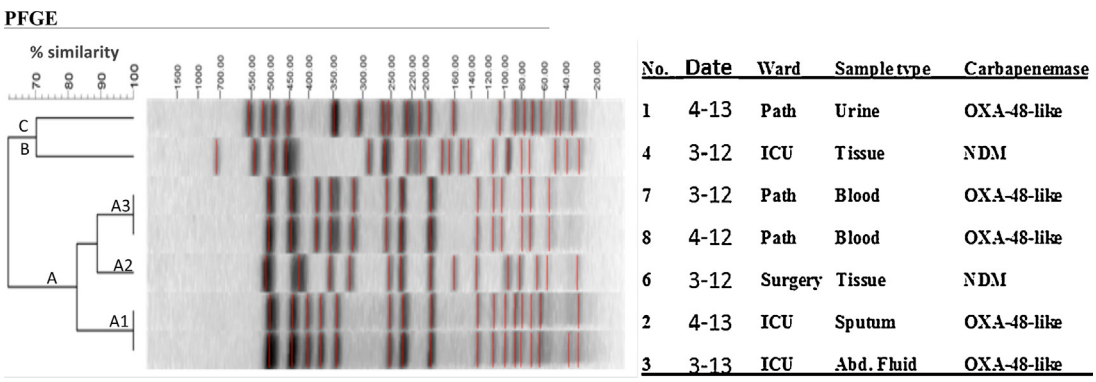
Please cite this article in press as: Aqel AA, et al. Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study. J Infect Public Health (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.002

Table 1 Metadata, antimicrobial susceptibilities and molecular characteristics of the seven carbapenemase producing *K. pneumoniae*.

Code No.[a]	Isolation date	Location	Specimen	Sex	Age	Carbapenemase and CTX-M gene families	PFGE group	Antibiotic susceptibility pattern[b]								
								FOX	CAZ	ATM	AMK	TET	SXT	TIG	COL	CARB
1 JO	13/04/2013	Internal medicine ward	Urine	M	75	*bla* OXA-48-like, $bla_{CTX\text{-}M\text{-}1\text{-}like}$	B	R	R	R	R	R	R	S	S	R
2 SYR	16/04/2013	ICU	Sputum	M	31	*bla* OXA-48-like, $bla_{CTX\text{-}M\text{-}9\text{-}like}$	A3	I	I	R	R	S	R	S	S	R
3 SYR	06/03/2013	ICU	Abdominal fluid	M	32	*bla* OXA-48-like, $bla_{CTX\text{-}M\text{-}9\text{-}like}$	A3	I	I	R	R	S	R	S	S	R
4 JO	27/03/2012	ICU	Tissue	M	30	*bla* NDM, $bla_{CTX\text{-}M\text{-}1\text{-}like}$	C	R	R	S	R	R	R	S	S	R
6 YEM	28/03/2012	Surgical ward	Tissue	F	36	*bla* NDM, $bla_{CTX\text{-}M\text{-}1\text{-}like}$	A2	R	R	R	R	S	R	S	S	R
7 YEM	24/03/2012	Internal medicine ward	Blood	M	20	*bla* OXA-48-like, $bla_{CTX\text{-}M\text{-}1\text{-}like}$	A1	R	R	R	S	R	S	S	S	R
8 JO	05/04/2012	Internal medicine ward	Blood	F	73	*bla* OXA-48-like, $bla_{CTX\text{-}M\text{-}1\text{-}like}$	A1	R	R	R	S	R	S	S	S	R

[a] JO, Jordanian; SYR, Syrian; YEM, Yemeni; M, male; F, Female; ICU, intensive care unit.
[b] FOX, cefoxitin; CAZ, ceftazidime; ATM, aztreonam; AMK, amikacin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; TIG, tigecycline; COL, colistin, CARB, carbapenem drugs (imipenem, doripenem, ertapenem, meropenem). (S)ensitive/(I)ntermediate/(R)esistant interpretations according to CLSI 2014.

Please cite this article in press as: Aqel AA, et al. Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study. J Infect Public Health (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.002



No.	Date	Ward	Sample type	Carbapenemase
1	4-13	Path	Urine	OXA-48-like
4	3-12	ICU	Tissue	NDM
7	3-12	Path	Blood	OXA-48-like
8	4-12	Path	Blood	OXA-48-like
6	3-12	Surgery	Tissue	NDM
2	4-13	ICU	Sputum	OXA-48-like
3	3-13	ICU	Abd. Fluid	OXA-48-like

Figure 1 The genetic relatedness of the seven CPK isolated in Amman, Jordan. Dendrogram of percentage relatedness in the PFGE banding patterns shows types A, B, and C, and the sub-types A1, A2, A3 (annotated) indicating the diversity present amongst the 2 NDM and 5 OXA-48-like producing *K. pneumoniae*. No., isolates numbers; Path, pathology department; ICU, intensive care unit; Abd, abdominal.

seek the detection of ESBL and/or carbapenemase phenotypes or genes [18].

In this first report of NDM and OXA-48-like CPK in Jordan we found isolates in multiple patients of three different nationalities, hospitalized in a single center. The two NDM-1 producers were classified into different PFGE types A2 and C. The type A2 isolate was from a Yemeni patient while the type C isolate was from a native Jordanian with no travel history, but both were hospitalized at the same time. The NDM positivity of these distinct isolates may reflect the horizontal transfer of an NDM encoding plasmid or the endemicity of NDM producing isolates in Jordan and Yemen. Indeed, international transfer of NDM gene(s) has been confirmed in Europe and the USA [19,20]. The five OXA-48 producers were classified into PFGE types A and B. Type B was represented by a single isolate from a Jordanian patient. Within PFGE type A, sub-types A1 and A3 each comprised two isolates. Isolates from subtype A3 were from two Syrian patients treated in ICU at different times, and may reflect repeated introductions of the bacterial strain by Syrian patients or by a lapse in infection control measures. The type A1 isolates were from, first a Yemeni patient, followed by a native Jordanian treated on the same ward only 12 days apart and may reflect the importance of strict implementation of infection prevention measures. CTX-1 and CTX-M-9 type ESBLs predominate amongst ESBL producers in Jordan [14] and they were found to be endemic amongst the OXA-48-like and NDM CPK we identified In terms of possible remaining treatment options for the MDR isolates we were able to confirm tetracycline as a possible treatment option for (42.8% of the isolates) while tigecycline and colistin both remained as solid treatment options against the carbapenemase producers we detected.

In summary, this pilot study aimed to determine the molecular epidemiology of carbapenemase producing *K. pneumoniae* that occur in Jordan. While we found some evidence for clonal spread of CPK in our center our data were predominated by distinct carbapenemase genes and unrelated bacterial strains and likely reflects the endemicity of such organisms in our patient population. Improved surveillance of resistance and the genetic determinants of resistance must now be a priority for multidrug resistant *Enterobacteriaceae* in Jordan and across our region. Adhering to extensive infection control measures in community and hospital-acquired infections will help disrupt the further spread of carbapenemase producing isolates among hospitalized patients in Jordan.

Conflicts of interest

Ellington M. has received financial support from Bruker Daltonics (GmbH) for attending a conference. PHE's AMRHAI Reference Unit has received financial support for conference attendance, lectures, research projects or contracted evaluations from numerous sources, including: Achaogen Inc., Allecra Antiinfectives GmbH, Amplex, AstraZeneca UK Ltd., Becton Dickinson Diagnostics, The British Society for Antimicrobial Chemotherapy (BSAC), Cepheid, Check-Points B.V, Cubist Pharmaceuticals, Department of Health, Food Standards Agency, Glaxo Smithkline Services Ltd., Henry Stewart Talks, IHMA Ltd., Merck Sharpe & Dohme Corp, Meiji Seika Kiasya Ltd., Momentum Biosciences Ltd., Nordic Pharma Ltd., Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd., Rokitan Ltd., Smith & Nephew UK Ltd., Trius Therapeutics, VenatoRx, and Wockhardt Ltd.

Please cite this article in press as: Aqel AA, et al. Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study. J Infect Public Health (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.002

Ethical approval

Not required.

Acknowledgment

Dr. Amin Aqel visited the Department of Microbiology, National School of Public Health, Athens, Greece as part of the observership program of ESCMID.

References

[1] Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol 2008;29:1099—106.

[2] Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 beta-lactamase-producing Enterobacteriaceae over a 11-year period, 2001 to 2011. Euro Surveill 2013;18(31), pii:20549.

[3] Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 2009;9:228—36.

[4] European Antimicrobial Resistance Surveillance System (EARSS). EARSS annual report 2013. European Centre for Disease Prevention and Control. Available at: http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/table_reports.aspx [accessed 12.04.15].

[5] Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of *Klebsiella pneumoniae* carbapenemases (KPC) resistance. Infect Drug Resist 2012;5:133—41.

[6] Zowawi HM, Sartor AL, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, et al. Molecular characterization of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in the countries of the Gulf cooperation council: dominance of OXA-48 and NDM producers. Antimicrob Agents Chemother 2014;58:3085—90.

[7] Wartiti MA, Bahmani FZ, Elouennass M, Benouda A. Prevalence of carbapenemase-producing enterobacteriaceae in a University Hospital in Rabat, Morocco: a 19-months prospective study. Int Arab J Antimicrob Agents 2012;2(3:4).

[8] Wadi J, Haloub N, Al Ahmad M, Samara A, Romman A. Prevalence of meropenem susceptibility among Gram-negative pathogens isolated from intensive care units in Jordan. Int Arab J Antimicrob Agents 2011;1(1:3).

[9] Kattan R, Liddawi R, Ghneim R, Siryani I, Al-Dawodi R, Abu-Diab A, et al. Emergence of *Klebsiella pneumonia* carbapenemase (*bla*KPC-2) in members of the Enterobacteriaceae family in Palestine. Int Arab J Antimicrob Agents 2012;2(2):4.

[10] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement. CLSI document, M100-S24. Wayne, PA, USA; 2014.

[11] Amjad A, Mirza IA, Abbasi SA, Farwa U, Malik N, Zia F. Modified Hodge test: a simple and effective test for detection of carbapenemase production. Iran J Microbiol 2011;3:189—93.

[12] Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. J Antimicrob Chemother 2004;54:735—43.

[13] Giakkoupi P, Tryfinopoulou K, Kontopidou F, Tsonou P, Golegou T, Souki H, et al. Emergence of NDM-producing *Klebsiella pneumoniae* in Greece. Diagn Microbiol Infect Dis 2013;77:382—4.

[14] Aqel AA, Meunier D, Alzoubi HM, Masalha IM, Woodford N. Detection of CTX-M-type extended-spectrum beta-lactamases among Jordanian clinical isolates of Enterobacteriaceae. Scand J Infect Dis 2014;46:155—7.

[15] Poirel L, Ros A, Carrër A, Fortineau N, Carricajo A, Berthelot P, et al. Cross-border transmission of OXA-48-producing *Enterobacter cloacae* from Morocco to France. J Antimicrob Chemother 2011;66:1181—2.

[16] Matar GM, Dandache I, Carrer A, Khairallah MT, Nordmann P, Sabra A, et al. Spread of OXA-48-mediated resistance to carbapenems in Lebanese *Klebsiella pneumoniae* and *Escherichia coli* that produce extended spectrum beta-lactamase. Ann Trop Med Parasitol 2010;104:271—4.

[17] Carrër A, Poirel L, Yilmaz M, Akan O, Feriha C, Cuzon G, et al. Spread of OXA-48-Encoding Plasmid in Turkey and Beyond. Antimicrob Agents Chemother 2010;54:1369—73.

[18] Arnold RS, Thom KA, Sharma S, Phillips M, Kristie Johnson J, Morgan DJ. Emergence of *Klebsiella pneumoniae* carbapenemase-producing bacteria. South Med J 2011;104:40—5.

[19] Birgy A, Doit C, Mariani-Kurkdjian P, Genel N, Faye A, Arlet G, et al. Early detection of colonization by VIM-1-producing *Klebsiella pneumoniae* and NDM-1-producing *Escherichia coli* in two children returning to France. J Clin Microbiol 2011;49:3085—7.

[20] Mathers AJ, Hazen KC, Carroll J, Yeh AJ, Cox HL, Bonomo RA, et al. First clinical cases of OXA-48-producing carbapenem-resistant *Klebsiella pneumoniae* in the United States: the menace arrives in the new world. J Clin Microbiol 2013;51:680—3.

Please cite this article in press as: Aqel AA, et al. Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study. J Infect Public Health (2016), http://dx.doi.org/10.1016/j.jiph.2016.02.002